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IMMUNOHISTOCHEMICAL STUDY OF CYTOKERATIN EXPRESSION IN SUBCOLUMNAR RESERVE CELLS OF HUMAN UTERINE CERVIX

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We have studied the cytokeratin expression in subcolumnar reserve cells of the human uterine cervix using the immunohistochemical method. Five commercially available cytokeratin antibodies (AE-3, M630, M717, LL002, and CAM5.2) were used to stain a normal uterine cervix. Endocervical columnar cells stained strongly with CAM5.2, which reacts with low molecular-weight keratins (simple epithelium-type). The stratified squamous epithelium stained strongly in all layers with M630, which reacts with high molecular-weight keratins (squamous-type). Subcolumnar reserve cells stained not only simple epithelium-type cytokeratin (CAM5.2) but also squamous-type cytokeratins (M630, LL002). This data presented so far indicates that subcolumnar reserve cells exhibit squamous characteristics. In addition, they also exhibit the cytokeratins typically found in non-stratified (simple) epithelia including endocervical columnar cells. This supports the notion that subcolumnar reserve cells have the potential to give rise not only to squamous epithelial cells but also to columnar cells.

Subcolumnar cells of the uterine cervix were earlier referred to as "reserve cells" by Carmichael and Jeaffreson (1) who thought them to be a reserve depot for the regeneration of the mucousforming epithelium. They also suggested that these reserve cells have a role in the development of squamous metaplasia, which may eventually lead to the development of cervical intraepithelial neoplasia (CIN) or invasive carcinoma of the cervix (8).

The origin of the reserve cells has still not been determined. Persisting embryonal cells (12), subepithelial stromal cells (17), monocytes (15), and immigrating ectocervical squamous cells (5) have all been suggested as being the progenitors.

Cytokeratin are a group of intermediate filaments characterized by their molecular weight and isoelectric pH, and form part of the cytoskeleton of all kinds of epithelial cells (13). There are at least 19 subtypes of keratins, with molecular weights ranging from 40 to 68 kilodaltons (13). Generally, a simple pattern of low molecular-weight keratins is found in non-stratified (simple) epithelia and more complex patterns of high molecular-weight keratins are typical of stratified, especially squamous cell epithelia (3, 13). Many studies have now shown that the keratin composition of cells varies in different cell types, and in different stages of differentiation or development (13, 14).

Thus, cytokeratins have proved to be efficient tools for characterizing the differentiation of a particular cell type and for establishing the origin of certain cells. Furthermore, specific cytokeratin patterns make it possible to discriminate squamous from glandular differentiation (3, 13).

To characterize the subcolumnar reserve cells and the various cell types lining the normal and metaplastic endocervix, we evaluated the immunohistochemical patterns of cytokeratin expression of those cells.

MATERIALS AND METHODS

Tissues: Uterine tissue was obtained at hysterectomy performed in 24 women for myoma at the University Hospital of Yamanashi Medical College. Cervical tissues were fixed in 95% alcohol and embedded in paraffin.

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Antibodies: A panel of monoclonal antibodies to cytokeratins were used in this study (Table 1). Monoclonal antibody AE-3 reacts with a broad spectrum of molecular weights of cytokeratin. Monoclonal antibody M630 reacts with high molecular-weight cytokeratin no. 1, 5, 10, 14. Monoclonal antibody M717 reacts with cytokeratin no. 6, 18. Monoclonal antibody LL002 reacts with cytokeratin no. 14. Monoclonal antibody CAM 5.2 reacts with low molecular-weight cytokeratin no. 8, 18, 19. The reactivity of these monoclonal antibodies is useful in paraffin-embedded cervical tissue (11, 18).

Methods: Cytokeratin staining was performed using a LSAB (labelled-streptavidinbiotin) kit (Dako). Sections were deparaffinized in xylene, rehydrated through alcohol, and then immersed in 3% hydrogen peroxidase in methanol for 10 min to block endogenous peroxidase activity. Sections were subsequently washed in phosphate-buffered saline (PBS), and normal goat serum was applied for 10 min to reduce non-specific antibody binding. The sections were incubated with the appropriately diluted primary antibodies, or with control normal mouse serum for 30 min at room temperature. Biotinylated goat anti-mouse IgG was used as the linker. After washing, the streptavidin complex was applied, stained with diaminobenzidine and then counterstained with hematoxylin. Three categories were used for the evaluation of immunohistochemical reaction: (++) strongly positive, (+) moderately positive, (+/−) weakly positive, and (−) negative.

RESULTS

Each of the antibodies used in this study reacted exclusively with the epithelial cells. In addition to the endocervical columnar epithelium and the ectocervical stratified squamous epithelium, we paid special attention to the subcolumnar reserve cells. We also detected some areas that exhibited squamous metaplasia. The staining pattern in the various components is summarized in Table 2.

The stratified squamous epithelium of the normal ectocervix did not stain with CAM5.2 (Fig. 1). AE-3 stained all layers strongly. M630 also stained all layers strongly (Fig. 2). M717 and LL002 stained all layers moderately.

The endocervical columnar cells of the normal endocervix stained strongly with CAM5.2 (Fig. 3), moderately with M717 and AE-3. However, they were negative to M630 (Fig. 4) and LL002. Subcolumnar reserve cells appear in a few of the normal cervical glands as a single layer of cuboidal cells beneath the columnar cells. These subcolumnar reserve cells were strongly labelled with M717 and M630 (Fig. 4), moderately with AE-3 and CAM5.2

TABLE 1. *Antibodies used for the study*

Antibody	No. in Moll's Catalog	Dilution	source
AE-3	1, 2, 3, 4, 5, 6, 7, 8	1 in 200	ICN Immuno Biologicals
M 630	1, 5, 10, 14	1 in 50	Dako
M 717	6, 18	1 in 50	Dako
LL 002	14	1 in 10	Novocastra
CAM 5.2	8, 18, 19	1 in 10	Becton-Dickinson

TABLE 2. *Reactivity of cytokeratin antibodies in endo- and ectocervical cells*

	AE-3	M 630	M 717	LL 002	CAM 5.2
Columnar cells	+	−	+	−	++
Reserve cells	+	++	++	+/−	+
Metaplastic cells	++	++	+	+/−	+/−
Squamous epithelial cells	++	++	+	+	−

(++) strongly positive
(+) moderately positive
(+/−) weakly positive
(−) negative

FIG. 1. Squamous epithelial cells show no reaction with CAM5.2. ×100

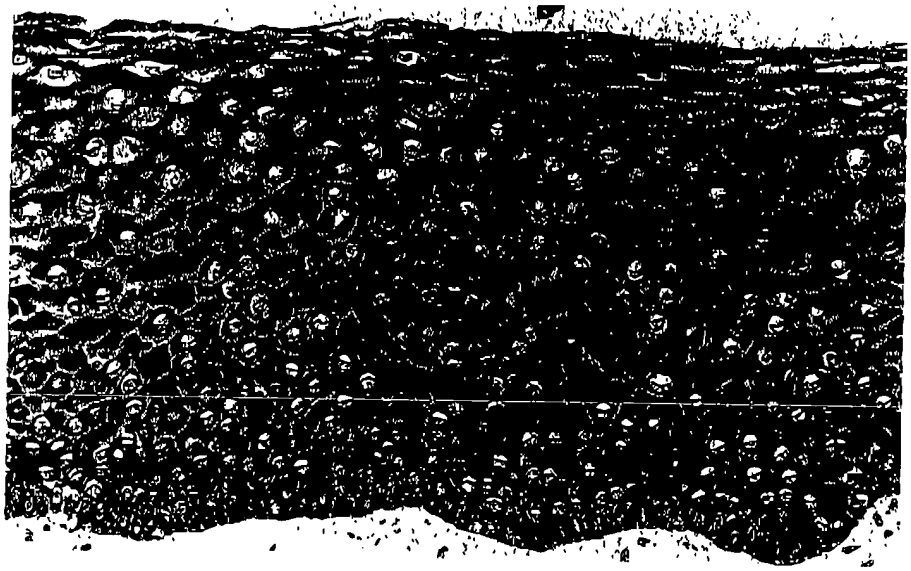

FIG. 2. Squamous epithelial cells stain strongly with M630. ×100

(Fig. 3), and weakly with LL002 (Fig. 5). The immunohistochemical staining produced by M630 allowed the easy identification of these cells even at low magnification. The staining also made it possible to achieve an unequivocal identification of even flat and inconspicuous subcolumnar reserve cells, which were difficult to detect or to distinguish from stromal cells.

The metaplasia examined in the endocervix was from the transformation zone and was of the immature type. The keratin expression of the metaplastic squamous cells is similar to that of ectocervix. However, these cells stained weakly with CAM5.2 (Fig. 6).

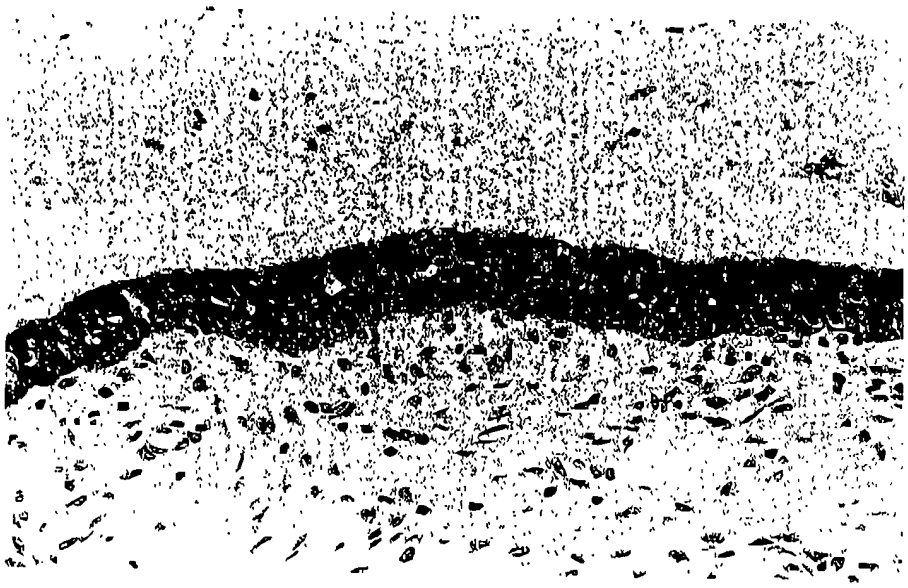

FIG. 3. Columnar cells stain strongly with CAM5.2. Subcolumnar reserve cells stain moderately. ×100

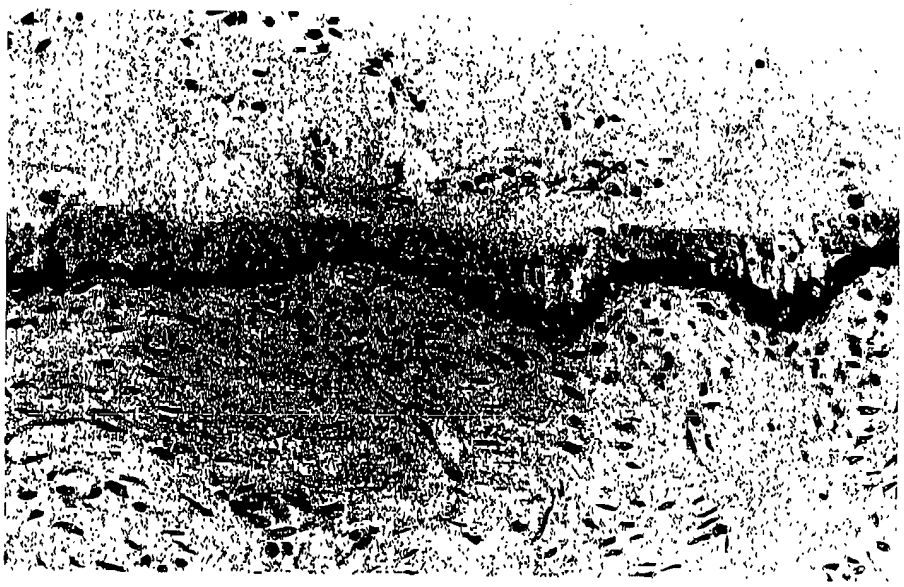

FIG. 4. Subcolumnar reserve cells stain strongly with M630. Columnar cells show no reaction. ×100

DISCUSSION

We analyzed the expression of cytokeratin in subcolumnar reserve cells of the human endocervical mucosa using a panel of antibodies directed against cytokeratins. We showed that the reserve cells expressed certain cytokeratins that were not detectable in endocervical columnar cells. Most of the previous investigations found a low incidence of subcolumnar reserve cells (4). In contrast to purely morphological studies, our immunohistochemical study facilitated the reliable identification of reserve cells, as evidenced by detection of such cells in 21 of the 24 uteri studied. This finding is an agreement with the im-

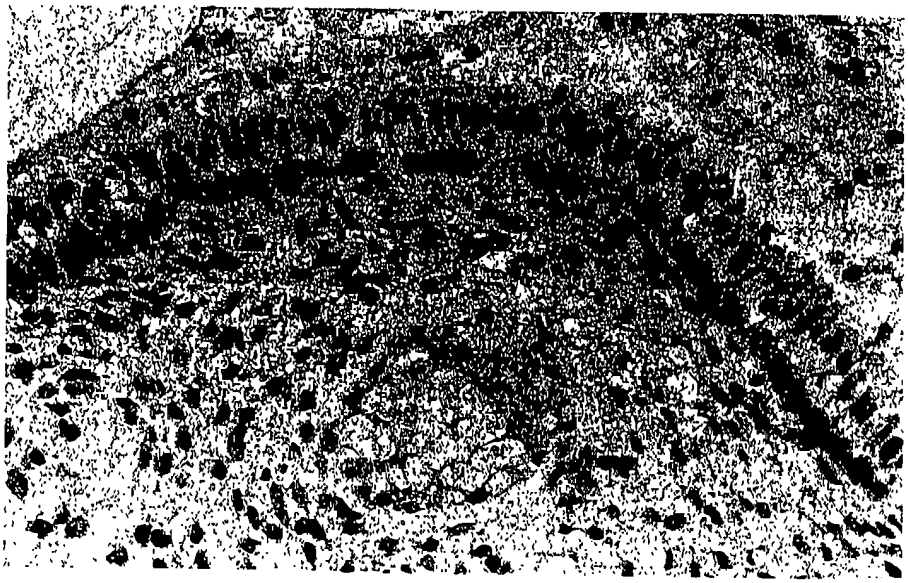

FIG. 5. Subcolumnar reserve cells stain weakly with LL002. ×150

FIG. 6. Metaplastic cells stain weakly with CAM5.2. Squamous epithelial cells show no reaction. ×150

munohistochemical results of Weikel (19), who found reserve cells in 100% of their samples.

Extensive studies of normal epithelia and carcinomas have shown that cytokeratins no. 5, 14 and 17 are consistently expressed in all normal stratified squamous epithelia and squamous cell carcinomas, but are absent from all non-stratified epithelia (3, 13, 14). Based on these studies, it has been suggested that those cytokeratins are general markers for squamous epithelia.

The keratin profile of subcolumnar reserve cells is in dispute. Weikel *et al.* (19) using broad spectrum antibodies, as well as antibodies to specific cytokeratin types, concluded that the subcolumnar reserve cells

contain cytokeratins no. 5 and 17, which are markers of squamous epithelia. Levy *et al.* (10) using antibodies to cytokeratins no. 13 and 18 found strong reactivity for keratin no. 13 in all reserve cells, but not to no. 18. Smedts *et al.* (16) using antibody LL002, RGE53, CAM5.2 and LPK showed that the subcolumnar reserve cells contain cytokeratin no. 5, 8, 18 and 19. In this study, subcolumnar reserve cells reacted with antibody M630 (cytokeratins no. 1, 5, 10, 14) very strongly and with antibody LL002 (cytokeratin no. 14) weakly. This is in accordance with the findings of Weikel *et al.* (19) and Ivanyi *et al.* (9) and supports the view that the reserve cells contain cytokeratin mainly found in squamous epithelia and undergo a squamous differentiation to a limited degree.

We showed that subcolumnar reserve cells also express some cytokeratins of the simple-epithelial type, as reacted with CAM5.2 (cytokeratin no. 8, 18, 19) moderately and with M717 (cytokeratins no. 6, 18) strongly. Weikel *et al.* (19) found no reaction in subcolumnar reserve cells with cytokeratin no. 18 antibody CK-2. In contrast, Gigi-Leitner *et al.* (6) showed a positive reaction for cytokeratin no. 18 in subcolumnar reserve cells with antibody Ks18. Smedts *et al.* (16) also showed a positive reaction for cytokeratin no. 18 in some of them with antibody PCK106. These discrepancies can be explained by presuming that these different cytokeratin no. 18 antibodies recognize different epitopes, or exhibit varying affinities for their respective antigens. As we and others have shown, these cytokeratins are expressed in various simple and glandular epithelia, including endocervical columnar cells (6, 13). This shows a relationship between the endocervical columnar and reserve cells. However, one should bear in mind that cytokeratin no. 19 is not strictly specific for simple epithelia since it also occurs in certain stratified squamous epithelia (9, 13, 16). The hypothesis that subcolumnar reserve cells originate from mesenchymally derived cells may be ruled out by a negative reaction for vimentin, and a positive reaction for cytokeratin (2, 9, 19). In view of the fact that certain simple epithelial cytokeratins are expressed in both columnar and reserve cells, there is a possibility that subcolumnar have the potential to give rise to columnar cells.

The cytokeratin profile of squamous metaplasia is characterized by its content of combinations of cytokeratins that differ from cervical columnar cells (6, 16). Gernow *et al.* (7) showed that differentiation into metaplastic squamous epithelium is followed by a loss of low molecular-weight cytokeratins and a high content of high molecular-weight cytokeratins. In squamous metaplastic epithelium we also found cytokeratin expression that corresponded to that found in squamous epithelium (high molecular-weight cytokeratin M630) and weak reacton with low molecular-weight cytokeratin CAM5.2. Our findings indicate that the reserve cells acquire a new property after the synthesis of squamous-epithelium type cytokeratin (M630). It seemed that squamous metaplasia developed via the proliferation of subcolumnar reserve cells that increasingly exhibit the characteristics of squamous epithelium (19). This study indicates that the subcolumnar reserve cells exhibit squamous characteristics. In addition, they also exhibit the cytokeratin typically found in simple epithelia including endocervical columnar cells. Indeed, our findings concerning their cytokeratin pattern emphasize the primitive yet dual squamous/simple-epithelial differentiation pattern of these cells. This supports the notion that reserve cells have the potential to give rise not only to squamous epithelial cells but also to columnar cells.

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